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Decl. w/attach
PATENT 7.23.03

Docket No. 273402004000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Jeffrey John GORMAN

Serial No: 09/202,035

Filing Date: December 17, 1998

For: VIRAL PEPTIDES WITH
STRUCTURAL HOMOLOGY TO
PROTEIN G OF RESPIRATORY
SYNCYTIAL VIRUS

Examiner: B. Li

Group Art Unit: 1648

**DECLARATION OF JOSEPH NOOZHUMUTRY VARGHESE
PURSUANT TO 37 C.F.R § 1.132**

Commissioner for Patents
Washington, D.C. 20231

I, Joseph Noozhumutry Varghese, declare as follows:

1. I am an employee of Commonwealth Scientific and Industrial Research Organisation (CSIRO), Division of Health Sciences and Nutrition, 343 Royal Parade, Parkville, Victoria 3052, Australia. I was appointed as a Principal Research Scientist by CSIRO in 1984 and currently hold the position of manager of CSIRO Health Sciences and Nutrition's Structural Biology Program.
2. I currently reside at 176 Nicholson Street, Brunswick East, Victoria, 3057, Australia.
3. My research interests over the past two decades have involved investigation of the structure and function of cell receptors, viral antigens and drug resistance in pathogenic organisms. I am skilled in the area of the development of antiviral agents for therapeutic

applications. My educational background and a list of my publications is set out in my curriculum vitae, a copy of which is attached as Annexure A.

4. I have read and am familiar with the contents of the United States patent application no 09/202,035 entitled: "Viral peptides with structural homology to protein G of Respiratory Syncytial Virus" ("the patent application").
5. The invention described in the patent application is based on the surprising finding that peptides derived from residues 149-197 of the G protein of respiratory syncytial virus (RSV) are capable of inhibiting the cytopathic effect (cpe) of RSV.
6. The "cytopathic effect" of a virus refers to morphological changes shown by cells in response to infection by that virus. For example, the cytopathic effect of a virus may consist of cell rounding, cell disorientation, cell swelling or shrinking, cell death etc. Cytopathic effect was a very well known phenomenon by June 1996 and methods for assessing the ability of an antiviral agent to inhibit a virus-induced cytopathic effect were in common use at that time. The following citations provide evidence that cytopathic effect assays were well known and used routinely in the field of virology by June 1996:
 - (i) McManus (1976) Microtiter assay for interferon: Microspectrophotometric quantitation of cytopathic effect. *Appl. Environ. Microbiol.* 31:35-38
 - (ii) Familletti *et al* (1981) A convenient and rapid cytopathic effect inhibition assay for interferon. *Methods Enzymol.* 78:387-394
 - (iii) Iwata *et al* (1996) Cytopathic effect inhibition assay for canine interferon activity. *J. Vet. Med. Sci.* 58:23-27.
 - (iv) Yousefi *et al* (1985) A practical cytopathic effect/dye-uptake interferon assay for routine use in the clinical laboratory. *Am. J. Clin. Pathol.* 83:735-740.

7. An example of a suitable assay for determining the impact of peptides on the cytopathic effect of RSV is described in the patent application at page 36, line 32 to page 37, line 9. Moreover, suitable alternative cytopathic effect assays would have been well known to those skilled in this field by June 1996.
8. The results presented in the patent application show that peptides derived from the non-glycosylated domain (defined by residues 149-197) of the G protein of RSV are capable of inhibiting the cytopathic effect of RSV.
9. The patent application also makes it clear that within this non-glycosylated domain, the region between residues 149-177 is particularly important in terms of inhibiting the cytopathic effect. For example, it is stated in the patent application at page 36, lines 5-13:

"The demonstration that Ac149-177 exhibited a profound enhancement of HEp-2 cell bound fluorescence, capping of cell bound fluorescence distribution shows that a binding interaction site is located in the region of the RSV G protein between residues 149-177. This surprising finding also indicates that the influence of the binding site of Ac149-177 does not depend on disulphide bonds, since the two cysteinyl thiols of this peptide derivative were protected by the acetamidomethyl group."

10. Based on the disclosure of the patent application, a person skilled in this area would be able to readily design compounds derived from the region of the RSV G protein between residues 149-197 and screen these compounds for inhibition of the cytopathic effect of RSV. Given that suitable screens have been available since before the filing date of the patent application, a person skilled in this field would be able to readily screen large numbers of candidate compounds without undue experimentation.
11. Indeed, I am aware that after the filing of the patent application, the inventor went on the test further peptides derived from this region of the RSV G protein. The results of this further research were published in 2001 (see Gorman *et al*, 2001, Antiviral activity and

Structural Characteristics of the Nonglycosylated Central Subdomain of Human Respiratory Syncytial Virus Attachment (G) Glycoprotein, J. Biol. Chem. 276:38988-38994, a copy of which is attached as Annexure C). The results presented in Figure 2 of this publication show that a number of different peptides derived from this regions were capable of inhibiting an RSV-induced cytopathic effect in human epithelial cells.

12. In view of the results presented in the patent application, a person skilled in this area would understand that compounds comprising peptides sequences derived from the region of the RSV G protein between residues 149-197 are capable of inhibiting RSV-induced cytopathic effect in human epithelial cells and are therefore suitable candidates for use in diagnostic, therapeutic and prophylactic methods for the prevention of RSV infection. The design and testing of suitable candidate compounds would be readily achieved by a person of ordinary skill in this field in light of the information provided in the patent application.
13. A person skilled in this area would also understand that methods for protecting against viral infectivity by inhibiting the virus-induced cytopathic effect are by their nature completely different to methods that involve immunisation or vaccination strategies. For example, inhibition of the cytopathic effect by antiviral agents may occur by interaction of the antiviral agent with the cellular receptor through which the virus normally attaches to the cell. In other words, the antiviral agent may itself bind to the cellular receptor thereby inhibiting attachment of the virus to the cell.
14. It would also have been known to a person of skill in this area that therapeutic or prophylactic regimes that involve the use of agents that inhibit virus-induced cytopathic effect would preferably involve topical administration of the antiviral agent. The topical administration may involve, for example, nasal inhalation.
15. In contrast, therapeutic or prophylactic regimes that aim to raise an immune response against a virus typically involve intravenous administration to a subject of viral antigens. Administration of the viral antigens triggers production by the subject of antibodies directed against specific regions of RSV.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

11/6/2003
Date

J. N. Varghese
Joseph Noozhumury Varghese

Serial No. 09/202,035
Docket No. 273402004000

CURRICULUM VITAE

Name: VARGHESE, Joseph Noozhumurry
Address: C.S.I.R.O. Division of Health Sciences & Nutrition
343 Royal Parade, Parkville

Academic Qualifications:

BSc(Hons) in Theoretical Physics, (1966-69) University of Queensland.
PhD in Physics (*Charge Density and Thermal Motion Analysis of Molecular Crystals*),
Department of Physics, (1970-73) University of Western Australia

Current Appointment: Head, Structural Biology, C.S.I.R.O. HSN

Professional Appointments:

1974-75: Senior Tutor/Demonstrator in Physics, University of Queensland
Classical and Quantum Mechanics
1975-78: Post Doctoral Fellow at the University of Western Australia.
Charge distribution in transition-metal complexes
1978-80: Lecturer, School of Molecular Sciences, the University of Sussex, UK.
Polarized neutron scattering and spin transfer
1980-84: Post Doctoral Fellow (1980-84), CSIRO Division of Protein Chemistry.
Structural Biology – Influenza Virus Neuraminidase Structure
1984-90: Principal Research Scientist, CSIRO Division of Biomolecular Engineering
Development of Relenza, Antibody-Antigen Complexes, Plant Storage proteins
1990-00: Seconded to the Biomolecular Research Institute, Chief Research Scientist in
Protein Structure Group.
Drug Resistance, Cytokines, Plant Glucanases and Protein Thermostability
2001- Head, Structural Biology Program CSIRO HSN
Scientific Management, Interleukin-6 Receptor Signalling, neurodegenerative
diseases

Summary of Current Research Interests and Highlights:

Contributed to the discovery of the anti-influenza drug Relenza (Zanamivir), and is a co-inventor on the international patent. Continued discoveries and authorship of seminal scientific papers (including four *Nature* articles) in the field of influenza neuraminidase research and inhibitor design. The design of Zanamivir was based on the structure of sialic acid complexed with influenza neuraminidase determined by Varghese.

Over the last two years assembled and led a multi-disciplinary team from the Ludwig Institute, CSIRO and B.R.I. to successfully purify and crystallise and solve the structure of the interleukin-6 receptor, which is an important frontline component of the body's armoury against infection and tissue damage. The work has now formed the basis for drug discovery program against a variety of human and has recently been published in PNAS and has generated several provisional patents.

Was responsible, through the use of X-ray structural studies, for the successful engineering of a glucanase enzyme from barley seed, to enhance its thermostability, in order to improve the malting quality of barley and holds an international patent on this.

Led the construction at the CSIRO structural laboratory of the brightest laboratory X-ray source for protein crystallography in the world and has placed the laboratory at the leading edge in X-ray data collection from small weakly diffracting protein crystals which has played a key role in the determination of Insulin like growth factor receptors, Epidermal Growth Factor receptors, Interleukin-6 receptor and a viral fusion protein. Responsible for the construction of

the 64-node Caduceus Beowulf Cluster, which is one of the fastest dedicated drug design facility in the world. Currently also leading a team at CSIRO who are constructing a Beowulf cluster to simulate proteins at a quantum mechanical level, to pursue an emerging interest in neuro-degenerative diseases.

In 2001 was invited by CSIRO to head the Structural Biology Program of the Division of Heath Sciences and Nutrition. The program consists of an X-ray diffraction laboratory, Drug Design Facility, Virology and Parasitology laboratories and a Proteomics Facility with a staff of 27 scientists, several doctoral and undergraduate students. Runs yearly workshops on structural biology for honours students for Melbourne Universities and has been on the education and training committee of the CRC-Cellular Growth Factors over the last ten years.

Had over twenty years experience in the structural biology aspects of synchrotron radiation both in instrumentation and data analysis, and is currently a member of the board of the Australian Synchrotron Research Program, and a member of the National Synchrotron Scientific Advisory Committee where he is championing the construction of two protein crystallography beamlines.

Publications last 5 years

1. JL McKimm-Breschkin, A Sahasrabudhe, TJ Blick, TJ McDonald, PM Colman, GJ Hart, RC Bethell, **JN Varghese**.
'Mutations in a conserved residue in the influenza virus neuraminidase active site decreases sensitivity to Neu5Ac2en derivatives'
(1998) **J. Virology** 72,2456-2462.
2. NR Taylor, A Cleasby, O Singh, T Skarzynski, A Wanacott, PW Smith, SL Sollis, PD Howes, PC Cherry, R Bethell, P Colman, **J Varghese**.
'Dihydropyranocarboxamides related to zanamivir - a new series of inhibitors of influenza virus sialidases - 2 - Crystallographic and molecular modeling study of complexes of 4-amino-4H-pyran-6-carboxamides and sialidase from influenza virus types A and B'
(1998) **J.Med.Chem.** 41(6),798-807.
3. **JN Varghese**, PW Smith, SL Sollis, TJ Blick, A Sahasrabudhe, JL McKimm-Breschkin, PM Colman.
'A structural basis for resistance to potent neuraminidase inhibitors in a variant of influenza virus'
(1998) **Structure** 6,735-746.
4. M Hrmova, **JN Varghese**, PB Høj, GB Fincher
*'Crystallization and preliminary X-ray analysis of β -D-glucan exohydrolase from Barley (*Hordeum vulgare*)'*
(1998) **Acta Cryst.** D54,687-689.
5. A Sahasrabudhe, L Lawrence, VC Epa, **JN Varghese**, PM Colman, JL McKimm-Breschkin,
'Substrate, inhibitor or antibody stabilizes the Glu 119 Gly mutant influenza virus neuraminidase'.
(1998) **Virology** 247,14-21.
6. **JN Varghese**, M Hrmova, GB Fincher.
'Three-dimensional structure of a barley 3 β -D-glucan exohydrolase, a family 3 glycosyl hydrolase'
(1999) **Structure**,7,179-190.
7. **JN Varghese**
'Development of neuraminidase inhibitors as anti-influenza virus drugs'
(1999) **Drug Devel.Research**,46,176-196.
8. AJ Harvey, M Hrmova, R DeGori, **JN Varghese**, GB Fincher

- 'Comparative modeling of the three-dimensional structures of Family 3 Glycoside Hydrolases'
(2000) **Prot.Science**, 41:257-269.
9. BJ Smith, PM Colman, M von Itzstein, B Danylee, JN Varghese
'Analysis of Inhibitor Binding in Influenza Virus Neuraminidase'
(2001) **Protein Sci.** 10/4:689-696.
 10. RJ Stewart, JN Varghese, TPJ Garrett, PB Hoj, GB Fincher
'Mutant barley (1-3,1-4)-B-glucan endohydrolases with enhanced thermostability'
(2001) **Protein Eng.** 14:245-253.
 11. M.Hrmova, JN Varghese, R. DeGori, BJ Smith, H Driguez, GB Fincher
'Catalytic mechanisms and reaction intermediates along the hydrolytic pathway of a plant β -D-glucan glucosylhydrolase; crystallographic, chemical and quantum mechanical analysis'
(2001) **Structure**, 9:1005-1016.
 12. Wyatt PG, Coomber BA, Evans DN, Jack TI, Fulton HE, Wonacott AJ, Colman P, Varghese J
'Sialidase inhibitors related to zanamivir. Further SAR studies of 4-amino-4H-pyran-2-carboxylic acid-6-propylamides'.
(2001) **Bioorg Med Chem Lett.** 11(5):669-73
 13. M.Hrmova, R. DeGori, BJ Smith, JK Fairweather, H Driguez, JN Varghese, GB Fincher
'Structural basis for a broad specificity in higher plant-beta-D-glucan glucosylhydrolases: kinetic, crystallographic and molecular modeling studies'
(2002) **Plant Cell** 14:1033-1052
 14. BJ Smith, JL McKimm-Breschkin, M McDonald, RT Fernley, JN Varghese, PM Colman
'Structural studies of resistance in influenza virus neuraminidase to inhibitors'
(2002) **J. Med.Chem.** 45:2207-2212
 15. JN Varghese, RL Moritz, M-Z Lou, A vanDonkelaar, H Ji, N Ivancic, KM Branson, NE Hall, RJ Simpson
'Structure of the extracellular domains of the human interleukin-6 receptor α -chain'
(2002) **Proc.Nat.Acad.Sci.USA** 99:15959-15964

International Patents

1. PCT/AU91/00161 "Derivatives and analogues of 2-deoxy-2,3-didehydro-N-Acetyl Neuraminic acid and their use as anti-viral agents"
M. von Itzstein, W-Y Wu, T H Phan, B Danylec, B Jin, P M Colman, J N Varghese.
2. PCT/AU94/00377 "(1-3,1-4)-b-glucanase of enhanced stability"
J N Varghese, T P Garrett, G Fincher, P Hoj, L Chen.

+ several provisional patents on cytokine signalling inhibitors, novel diagnostics and anti-virals